

phosphine oxide (6 mg). The resulting solution was concentrated to a thin film in vacuo overnight. Swell buffer (TRIS 50 mM sodium acetate 50 mM, sodium chloride 50 mM, 10^{-7} M europium nitrate, pH 7.0) was added and the flask warmed at 50° to 60° C. to suspend the lipids. The mixture was sonicated with a titanium probe (75 watts, 5×2 min) cooling with ice water between each sonication. The mixture was then centrifuged at low speed to remove titanium particles and extruded through polycarbonate membranes (5, 0.4, and 0.2 μ). This mixture was then passed over a sephadex column (G-25, med, 2×30 cm) previously equilibrated and eluted with coupling buffer (TRIS 50 mM, sodium acetate 50 mM, sodium chloride 50 mM, 10^{-7} M europium nitrate, pH 8.0). The fluorescent fractions were collected (5 ml/ea), and the two most fluorescent fractions combined. The liposome suspension was then combined with a first monoclonal anti-hCG antibody (2 mg) previously reduced with dithiothreitol (DTT) and purified on sephadex G-25 med. using coupling buffer as eluent. This mixture was allowed to stand overnight in the dark at ambient temperature, then purified by centrifugation (75,000 Xg, 0.5 h) twice in storage buffer (TRIS 50 mM, sodium acetate 50 mM, glycerol 2%, DMSO 0.5 mL/L sodium azide 0.2 g/L, 10^{-7} M europium nitrate, pH 7.4, milliosmolality 310). The liposomes were stable for at least one month at 4° C.

2. Microtiter Strips

A solution of a second monoclonal anti-hCG antibody (1.76 μ g/mL) in coating buffer (sodium carbonate 1.59 g/L, sodium bicarbonate 2.93 g/L, thimerosal 0.1 g/L pH 9.6) was aliquoted into microtiter strips (200 μ L/well, Titretrek), covered, and allowed to stand at 4° C overnight. The strips were then washed with phosphate buffered saline (3X). TRIS blocking buffer (TRIS 50 mM, sodium chloride 9 g/L, BSA 0.5%, sodium azide 0.2 g/L, pH 7.7; 300 μ L/well) was added and the strips allowed to stand at 4° C. overnight. The blocked strips were washed with HEPES assay buffer (HEPES 50 mM, sodium chloride 9 g/L, BSA, 0.5%, sodium azide 0.2 g/L, pH 7.7; 200 μ L/well), and stored humidified at 4° C.

3. Assay Procedure

Serum standards of hCG (LKB, Gaithersburg, MD) were aliquoted into microtiter strips (20 μ L/well) and diluted with HEPES assay buffer (200 μ L/well). The strips were shaken for 1.5 h, and washed with HEPES assay buffer (6X). A solution of liposomes (250 μ L) in HEPES assay buffer (10 mL) was added (200 μ L/well) and the strips shaken 1.25 h. The strips were washed with HEPES assay buffer (6X) and read in an Arcus (LKB) fluorometer. A standard curve plotting concentration versus fluorescence was constructed in which the linear range extended from 10 to 5000 mIU hCG per mL. The correlation of expected and observed values was 94%. The curve is shown in the Figure.

EXAMPLE II

Cell Labeling With Fluorescent Europium Liposomes

A suspension of hybridoma cells which express cell surface antibody to PC was adjusted to 5×10^6 cells/mL in Hank's balanced salt solution (HBSS). Serial dilutions of this suspension were placed into V-bottom polystyrene microtiter strips. A solution of fluorescent europium liposomes (example 1, 1/40 in HBSS, 50 μ L/well) was added. In the same fashion, control lym-

phoma cells which do not express the surface antibody to PC were run. The strips were washed twice by centrifugation with HBSS and then examined under uv light. The visual detection of 300 cells expressing surface antibody to PC was possible. None of the control cells was fluorescent. Further experiments using the same procedure demonstrated the ability to visually detect 1200 cells with specific antibody to PC in the presence of 10^4 non-specific control cells. The results were easily documented photographically using Polaroid film and a red filter (high pass at 610 nm). The experiment was again repeated with liposomes loaded with carboxyfluorescein. The strips were excited with uv light and viewed through a filter (500 nm high pass). In this case, it was not possible to determine specific cell binding due to background fluorescence of the polystyrene plate.

EXAMPLE III

Entrapment of A Water-Insoluble Perylene Dye in the Liposome Bilayer and Use as a Reagent for Cell Sorting

Phosphatidylcholine (94 mg), phosphatidylglycerol (10.3 mg), cholesterol (50.9 mg) and N,N'-bis(2,5-di-tert-butyl-phenyl)-3,4,9,10-perylene-carboximide (1.5 mg, Aldrich, Milwaukee, Wis.) were dissolved in chloroform and dried to a thin film. Storage buffer was added, and the mixture warmed, sonicated and extruded through polycarbonate membranes in a similar fashion to example 1. Cells expressing surface antibody to PC were allowed to stand in contact with the liposomes for about 15 min. at 0° C. These labeled cells were then placed on an Epics cell sorter (Coulter Diagnostics, Hialeah, Fla.) using excitation at 488 and a 595 high pass filter. Cells which had surface antibodies to PC were clearly distinguished from control cells without surface antibodies to PC.

EXAMPLE IV

Preparation of Phosphorescent Liposomes

Liposomes were prepared with N,N'-bis(2,5-di-tert-butyl-phenyl)-3,4,9,10-perylene entrapped in the lipid bilayer according to Example III. Phosphorescence was measured on a Spex fluorometer (excitation: 466 nm; emission: 621 nm; delay: 0.01 milliseconds; window: 0.2 milliseconds) using 5 nm slits and 100 flashes per point. At an approximate overall perylene concentration of 10^{-5} M, the phosphorescence measured 518,000 counts over a buffer background of 17 counts.

EXAMPLE V

Preparation of Liposomes Having Both a Fluorescent Dye Encapsulated in the Internal Aqueous Compartment and Entrapped in the Lipid Bilayer

A solution of PC (94 mg), phosphatidylglycerol (10.3 mg), cholesterol (50.9 mg), phosphatidylethanol-amine-maleamide reagent (3.75 mg), tri-n-octylphosphine oxide (6 mg), and tris-(b-naphthoyl)trifluoromethylacetylacetonato-bis-piperazine europium III complex (6 mg) in chloroform is dried to a thin film under reduced pressure. To this is added an aqueous solution (20 ml) of tetrasodium 4,7-bis-(4-phenylsulfonato)-1,10-phenanthroline-2,9-dicarboxylate (50 mM) and europium nitrate (50 mM) adjusted to pH 7.0 with NaOH. The mixture is warmed at 45° C and gently agitated to suspend the lipids. The suspended lipids are